DR. MARIANA LORENA TELLECHEA (Orcid ID : 0000-0002-1194-8433)

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Title: "Perinatal taurine exerts a hypotensive effect in male Spontaneously Hypertensive Rats and down-regulates endothelial Oxide Nitric Synthase in the aortic arch".

Authors and affiliations: Melisa F Mensegue[12], Adriana L Burgueño[3], Mariana L Tellechea[124¶]

[1] University of Buenos Aires. Institute of Medical Research A. Lanari. Ciudad Autónoma de Buenos Aires, Argentina.

[2] National Scientific and Technical Research Council (CONICET). Institute of Medical Research (IDIM). Department of Molecular Genetics and Biology of Complex Diseases. Ciudad Autónoma de Buenos Aires, Argentina.

[3] Instituto de Investigaciones Biomédicas. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Pontificia Universidad Católica Argentina. Ciudad Autónoma de Buenos Aires, Argentina.

[4] Present address: Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE), CONICET – FEI – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

¶ Corresponding author at: Gallo 1330, (C1425EFD) Buenos Aires, Argentina. E-mail address: mtellechea@cedie.org.ar

Short title: "Perinatal taurine in Spontaneously Hypertensive Rats"

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Abstract

Essential hypertension is considered to be a result of the interaction between genetic and environmental factors, including perinatal factors. Different advantageous perinatal factors proved to have beneficial long-lasting effects against an abnormal genetic background. Taurine is a ubiquitous sulphur-containing amino acid present in foods such as seafood. The antihypertensive effects of taurine have been reported in experimental studies and in human hypertension. We aimed to investigate the effects of perinatal treatment with taurine in spontaneously hypertensive rats (SHR), a known model of genetic hypertension. Female SHR were administered with taurine (3 g/l) during gestation and lactation (SHR-TAU). Untreated SHR and Wistar-Kyoto rats (WKY) were used as controls. Long lasting effects in offspring were investigated. Addition of taurine to the mother's drinking water reduced blood pressure in adult offspring. No differences were observed in cardiac hypertrophy. Findings on morphometric evaluations suggest that perinatal treatment with taurine would be partially effective in improving structural alterations of the aorta Modifications in gene expression of Bcl-2 family members and upregulation of endothelial nitric oxide synthase in the aorta of 22-week-old male offspring were found. No differences were observed on relative telomere length in different cardiovascular tissues between SHR and SHR-TAU. Altogether results suggest that taurine programming, albeit sex-specific, is associated with gene expression changes which ultimately may lead to improvement of aortic remodelling and enhanced endothelial function because of augmented nitric oxide (NO) production.

Keywords: hypertension; taurine; spontaneously hypertensive rats, foetal programming, endothelial nitric oxide synthase, telomere length

Introduction

Although the hereditary nature of cardiovascular diseases is well established, the perinatal origin of adult disease or programming hypothesis has been widely supported by many lines of evidence in animal models and human epidemiologic studies(1). Aberrant environmental factors acting early in life, in particular in the intrauterine and early postnatal periods,

correlate with arterial hypertension and cardiovascular disease later in life(2,3). Conversely, advantageous perinatal factors may have beneficial long-lasting effects against an abnormal genetic background. Different strategies have been used during pregnancy and lactation to ameliorate the development of hypertension in offspring in Spontaneously Hypertensive Rats (SHRs)(4–8), a widely used genetic model of human essential hypertension. Despite the genetic predisposition, the perinatal environment seems to play a role in the development of blood pressure in SHR.

Taurine (2-aminoethanesulphonic acid) is present in the diet and can also be synthesized from cysteine and methionine in the postnatal life. Numerous intracellular and extracellular functions have been described for taurine(9–11). Beside its antioxidant activity, which has long been recognized(12), it has been demonstrated that taurine acts as an anti-inflammatory agent(13,14). Taurine has been shown to have a number of essential biological functions *in vivo*. Although taurine is best known for its role in lipid metabolism, is also beneficial against a variety of aging-related diseases, such as chronic heart failure, diabetes, atherosclerosis, etc(15).Several groups have shown that addition of taurine to the drinking water of SHR had an effect on the development of hypertension(16–20). Furthermore, in two double-blind, placebo-controlled studies, oral administration of taurine decreased blood pressure in borderline hypertensive subjects compared with the placebo-treated subjects(21,22). In addition, taurine supplementation reduced carotid intima-media thickness of pre-hypertensive participants(19).The beneficial effects of taurine on hypertension, both in rat experimental models and in cases of human hypertension, have been reviewed elsewhere(23). Taurine may be an attractive and cost-effective approach to treat hypertension even during pregnancy. Furthermore, taurine-rich food is easily consumed daily. Some studies suggested that taurine has a programming effect on hypertension(24,25). The purpose of the present study was to determine whether taurine administration during pregnancy and lactation would influence 1) systolic blood pressure (SBP), 2) aortic geometry, and 3) cardiac hypertrophy in adult (22 weeks old) SHR. Additionally; we aimed to determine whether perinatal taurine administration is associated

with changes in relative telomere length (RTL) and gene expression of target genes at 22 weeks of age.

Results

Obstetric-neonatal outcomes

There were no differences in maternal body weight during pregnancy and lactation [Supplementary Information (SI) 1A] or in obstetrical-neonatal outcomes (SI 1B) between SHR and SHR-TAU groups. Perinatal taurine supplementation had no effect on postnatal growth (SI 1C).

Body weight in male and female offspring

Body mass was recorded during 19 weeks and no statistical differences were found between SHR and SHR-TAU groups in males and females (SI 2).

Systolic Blood Pressure

As expected, SBP was found to be elevated in SHR with respect to Wistar-Kyoto (WKY) rats ($P<0.0001$ in males and females, Figure 1). Repeated measures ANOVA showed that both male and female offspring from mothers on taurine achieved significantly lower SBP (Figure 1). Planned comparisons were performed and analysis detected decreased SBP in SHR-TAU with respect to SHR in both male and female especially between weeks 19 and 22.

Geometry of the thoracic aorta

Representative images of transversal aortic slices stained with hematoxylin-eosin are shown in SI 3. In line with previous data(26,27), hypertension in SHR led to an increase in the cross-sectional area (CSA) of the wall, a thickening of the wall (Wt) and the consequent increase in the Wt/lumen ratio (Table 1). Analysis of the effects of perinatal taurine revealed a tendency toward statistical significance ($P= 0.06$) in CSA values in males (Table 1). Additionally, CSA/BW values were found to be significantly reduced in aortas from SHR-TAU with respect to SHR also in males.

Cardiac and renal hypertrophy

As expected(26,28,29), we found left ventricular hypertrophy in SHR, as heart-to-body weight ratio was increased in comparison with WKY in both male [SHR 0.37± 0.02 (n=7) vs. WKY 0.35±0.01 (n=9), $P= 0.02$] and female rats [SHR 0.39± 0.01 (n=9) vs. WKY 0.33±0.03

(n=7), P< 0.001]. Perinatal taurine had no effect on the hypertension-induced cardiac hypertrophy in mature SHR [male SHR-TAU 0.384±0.01 (n=9) and female SHR-TAU 0.38±0.01 (n=11)]. The kidney-to-body weight ratio was not different between groups [males: SHR 0.37±0.02 (n=7), SHR-TAU 0.38±0.01 (n=9), WKY 0.35±0.01 (n=9); females: SHR 0.39±0.01 (n=9), SHR-TAU 0.38±0.01 (n=11), WKY 0.33±0.03 (n=7)].

Mitochondrial DNA content in the left ventricle

It has been suggested that the increase in mitochondrial mass and the mitochondrial DNA (mtDNA) content are early molecular events of human cells in response to endogenous or exogenous oxidative stress(30). It has also been demonstrated in SHR that the total number of copies of mtDNA increases as a function of age in the heart(31). Programming of oxidative stress in the left ventricle was investigated by measuring mtDNA content (Figure 2). We found no differences between groups on mtDNA content in the left ventricle at weaning or at 22-weeks old. Further analysis detected an effect of age (4 vs. 22- weeks old) in SHR, SHR-TAU and WKY (P<0.00001 in all groups).

Relative telomere length in cardiovascular tissues

We hypothesized that perinatal taurine would decrease the oxidative stress and/or the hemodynamic stress associated with hypertension, and therefore would prevent senescence. As mechanical shear forces induced by blood flow may play different roles in the process of vascular remodelling in different vessels, we selected the aortic arch and the iliac arteries as conductance (elastic) arteries, and the mesenteric arteries as small resistance (muscular) arteries. Perinatal taurine had no significant influence on RTL in cardiovascular tissues at adulthood (Figure 3). We determined RTL in aorta and iliac arteries at pre-hypertensive age and during established disease (Figure 3). Analyses showed no differences between age-matched groups. We further analysed the effect of age on RTL (4 vs. 22-wo). Only in males and especially in SHR, analysis detected decreased RTL in aortic arch with increasing age (P= 0.01). We have also studied RTL in mesenteric arteries; left ventricle and kidney at 22-wo but no differences were detected between groups (Figure 3). Finally, analysis was performed to assess RTL differences between tissues. RTL was similar in aortic arch and in iliac arteries in all groups (SHR, SHR-TAU and WKY) at two time points,

4 and 22-wo (SI 4). RTL was found to be increased in iliac arteries, and also in the aortic arch, with respect to mesenteric arteries in WKY and SHR-TAU at 22-wo, but not in SHR where no differences in RTL were found, suggesting decreased intrinsic cell turnover in resistance arteries in SHR. RTL in left ventricle was decreased with respect to kidney in all groups at 22-wo.

Gene expression in the aortic arch and in the left ventricle

We examined whether the expression of telomerase reverse transcriptase (Tert) was influenced by perinatal taurine in the aorta at 4 (pre-hypertensive stage) and 22 weeks after birth (Figure 4). The expression of mRNA Tert in SHR was not different to WKY. However, specifically in males, the expression of Tert was increased in the aorta of 22-wo SHR-TAU (P= 0.02). Programming of aortic senescence was investigated by measuring cyclin-dependent kinase inhibitor 2A (p16Ink4a) mRNA expression(32). It has been previously hypothesized that hypertension-induced increases in p16INK4a expression could result in a widespread irreversible cell cycle arrest with accumulation of senescent cells(28). Analysis showed no significant differences between groups (Figure 4).

An altered balance between proliferation and death has been proposed to be a determinant of vascular, renal, and cardiac remodelling in genetic hypertension(33–35). We were then intended to unravel if perinatal taurine may lead to changes in mRNA expression of BCL2-associated X protein (Bax) and B-cell leukemia/lymphoma protein-2 (Bcl-2) in the aortic arch as a consequence of alteration of the perinatal environment (Figure 4). Results showed that the Bax/Bcl-2 mRNA ratio was decreased in male SHR compared to male WKY (P= 0.0006). Interestingly, the effects of perinatal taurine were significant but in different directions in male (P= 0.0003) and female (P= 0.02) offspring. To further explore this sexual dimorphism, we measured the expression of p53 tumor suppressor (p53) in both SHR and SHR-TAU groups (Figure 4), and analysis detected a marked sexual dimorphism in the levels of p53: p53 mRNA expression was found to be higher in male (P= 0.006) and lower in female SHR-TAU aortic homogenates (P= 0.02).

We then aimed to investigate if perinatal taurine may program enhanced NO bioavailability in vessels by increasing endothelial nitric oxide synthase (eNOS) expression (Figure 4).

Planned comparisons indicated a down-regulation of eNOS in male SHR with respect to WKY in adulthood (P= 0.004). Taurine supplementation during gestation and lactation in SHR increased the transcript levels of eNOS in males (P= 0.01). The mRNA expression of antioxidant enzymes was measured to indirectly assess changes in the oxidative status programmed by perinatal taurine (Figure 4). Among the different antioxidant molecules, SOD and catalase mutually function as important enzymes in the elimination of reactive oxygen species. A differential expression between SHR and WKY in 22-wo aorta was detected only in the levels of superoxide dismutase 3 (SOD3 or EC-SOD). The transcript level of SOD3 was significantly downregulated at 22-wo in female SHR (P= 0.03). On the other hand, perinatal taurine had no effect on the expression of superoxide dismutase 2 (SOD2 or Mn-SOD), SOD3 or catalase.

Differences between groups at weaning failed to achieve statistical significance (data not shown, N male / female 4-wo offspring: 6-7 / 4 SHR, 5-8 / 5-9 SHR-TAU, 5-7 / 5 WKY).

Perinatal taurine had no significant influence on gene expression of Tert, p16Ink4a, Bax/Bcl-2, SOD2, SOD3 or catalase in the left ventricle in SHR in adulthood (SI 5).

Discussion

Our research shows that maternal taurine supplementation (3 g/l) during pregnancy and lactation is associated with a reduction in SBP in adult offspring. Of note, although treatment with taurine significantly reduced SBP in SHR, it remained elevated compared to that in WKY rats.

Findings on morphometric evaluations suggest that perinatal treatment with taurine, when initiated in utero, would be partially effective in improving structural alterations of the aortic artery. Decreased cardiac(17,20,36) and renal(20) hypertrophies in taurine-treated SHRs has been previously demonstrated, reinforcing the notion that the blood pressure- lowering effect of taurine also led to a decrease in hypertension-induced organ hypertrophy. However, according to our results, perinatal exposure to taurine has no benefits on end organ structure of the heart and kidney in SHR. In accordance, it was formerly reported that perinatal exposure to L-arginine and antioxidant supplements had no effect on kidney hypertrophy in SHR(4).

Several studies have shown that senescence can be triggered either by telomeric DNA instability resulting from telomere erosion (replicative senescence) or following exposure to multiple types of stress (stress-induced senescence), such as oxidative stress and DNA damage(37). We have anteriorly shown through meta-analysis that leukocyte telomere length (LTL), a novel biomarker for age and age-related diseases, is shorter in hypertensive than in normotensive individuals(38). Because offspring had no contact with taurine from the time point of weaning, changes in SBP represents long lasting effects. We proposed that exposure to taurine in the intrauterine and postnatal period may lead to permanent modifications such as changes in telomere length in target tissues. RTL in cardio-vascular tissues from perinatally supplemented SHR showed no difference with respect to control SHR. However, according to our results, maternal taurine treatment was associated with an increase in Tert gene expression in aorta, at least in males at 22-wo. Some studies have shown effects of taurine on telomere length, e.g. the average RTL measured by RT-PCR in livers of aging mice was positively correlated with blood levels of taurine(39). In rabbits, taurine treatment diminished the intimal hyperplasia associated with oxidative stress in iliac arteries, while also partially reversed the decrease in telomere shortening(37). Telomere size may play a prominent role in the etiology of arterial vascular remodelling in genetic hypertension; however, we were not able to detect changes in RTL or in the transcript levels of Tert in SHR.

Growth, apoptosis, low-grade inflammation, and vascular fibrosis are all dynamic processes that have been invoked to contribute to arterial remodelling in hypertension(40). Xiao F et al have reported increased Bcl-2/Bax mRNA and protein ratios in the aortic vascular smooth muscle cells (SMCs) of SHR aged 16-wo(41). However; data about Bax and Bcl-2 in the aorta are scarce and potentially controversial(38). It has been suggested that antihypertensive therapy may contribute to regression of vascular wall growth via activation of pro-apoptotic mechanisms(26,40,42).The correction of aortic SMC hyperplasia may contribute to the normalization of mechanical properties of the vessel wall, therefore restoring vascular compliance of conductance arteries(42). Accordingly, increased Bax/Bcl-2 ratio in aorta from SHR treated with enalapril or amlodipine in comparison to untreated SHR

was previously described(26). Furthermore, apoptosis in SMCs is enhanced transiently during the regression of aortic hypertrophy in SHR treated with different classes of antihypertensive drugs(43,44). Losartan induced a synchronous wave of apoptosis in SMCs, as evidenced e.g. by a transient increase in Bax/Bcl-2 ratio(45). In the present study, both Bax/Bcl-2 ratio and p53 were increased in SHR-TAU males, suggesting that the beneficial effect on blood pressure of perinatal supplementation with taurine in male SHR may be in part a result of vascular hypertrophy regression. In females, exposure to taurine from conception until weaning has shown to decrease both Bax/Bcl-2 ratio and p53 expression in the aortic arch. The mechanisms of the sexual dimorphism involved in the observed changes in Bax/Bcl-2 mRNA ratio and p53 mRNA are unclear; however, beyond marked sexual dimorphism, perinatal taurine through programming would prevent the decrease in Bax/Bcl-2 ratio in SHR. In a recent study, Liang W et al reported that 3% taurine attenuated the progression of vessel wall injury, and promoted the apoptosis of vascular SMCs via up-regulation of Bax protein and down-regulation of Bcl-2 protein(46). Further analyses are necessary to study the role of perinatal taurine on apoptosis in arterial walls.

Nitric oxide (NO), generated within vascular endothelium by eNOS, is the principal agent that regulates blood pressure by causing relaxation of vascular SMCs in conduit arteries(47). Our results were in accordance with previous reports. The expression of eNOS transcript in aorta of 25-wo male SHR, tested by quantitative RT-PCR, has previously probed to be decreased with respect to WKY(48). Concerning the mechanism through which perinatal taurine may act it is surely multifactorial, nonetheless putative mechanisms whereby perinatal taurine improve oxidative stress may be due to 1) an ability to regulate NO synthases that generate NO; 2) an ability to regulate enzymes such as NAD(P)H oxidase that generate free radicals; or 3) an ability to regulate antioxidant enzymes, including SODs, which metabolize free radicals. In the present study, eNOS gene expression in the aorta was affected by perinatal taurine, suggesting that this sulphur amino acid may increase NO production, thereby contributing to the prevention of endothelial dysfunction and hypertension. A beneficial effect of taurine has been formerly described in aortas from diabetic and protein restricted rats (49). Future functional studies should assess the effect of

perinatal taurine supplementation on the endothelium-dependent vasodilation. On the other hand, our results revealed that the beneficial effects of taurine supplementation appear not to be related to programmed antioxidant activity. Of note, in the present study perinatal taurine has shown to up-regulate both Tert and eNOS gene expression. A relationship between Tert expression and activity and eNOS/NO activation has been previously described(50–52).

The present study has some limitations that should be taken into consideration before drawing conclusions. The failure to reach significance in secondary outcomes could possibly be a type 2 error due to the limited sample size and sex-specific effects. Some maternal effects seem to be sex specific, suggesting a possible role of sex hormones, particularly oestrogen, in these effects. It was previously demonstrated that taurine can promote the secretion of prolactin, follicle-stimulating hormone and luteinizing hormone in female rats, also stimulating the levels of oestrogen and progesterone (53). Previous studies have shown gender-specific effects of maternal taurine supplementation (54–58). Koeners MP et al have found a more pronounced antihypertensive effect of perinatal treatment with a mixture of micronutrients, including taurine, in male than in female genetically hypertensive rats (58). It has been hypothesized that oxidative stress may not be as important in mediating hypertension in female as in male rats (59). Further studies of the mechanisms underlying sexual dimorphism are of substantial significance for the development of gender-specific strategies to ameliorate or prevent disease later in life. On the other hand, since alterations in mRNA levels may not necessarily reflect changes in corresponding proteins, further studies are required to hypothesis testing. Finally, through the current study design it is not possible to determine if the observed effects are a consequence of the decrease in maternal blood pressure during pregnancy and lactation or a consequence of direct or indirect effects on the fetus or on pre-weaning offspring. Maternal blood pressure certainly could play a role. Available data suggest that the hypotensive effect of taurine does not occur through one specific mechanism but rather through multiple mechanisms(22). On the other hand, it is also possible that taurine would have a beneficial effect on other maternal outcomes. More experimental studies are required to unveil the mechanisms resulting in blood pressure

programming. Taurine supplementation has been proposed to play a role in mediating inflammatory processes in SHR(60). It is therefore possible that the beneficial effects of perinatal taurine may be also attributed to anti-inflammatory actions on maternal or neonatal metabolic parameters.

Although SHR have responded only moderately to perinatal taurine treatment, our findings supports the possibility of improving the genetically hypertensive state by manipulating the early environment. In males, the blood pressure lowering effects of taurine supplementation appear to be related, at least in part, to the upregulation of eNOS and increased programmed apoptosis. Because of the long-lasting effect of maternal taurine treatment on gene expression, our future directions are to examine for epigenetic changes that are influenced by perinatal taurine. A recent study has shown that maternal treatment of SHR with pentaerythritol tetranitrate during the pregnancy and lactation periods leads to a persistent blood pressure reduction in the offspring, which was associated with upregulation of eNOS, SOD2, GPx1, and HO-1 and modifications in the promoter regions of eNOS, SOD2 and HO-1genes(8).

Unravelling the plethora of mechanisms resulting in the programming phenomenon could lead to simple maternal interventions aimed to ameliorate both increasing prevalence of common diseases and public health costs.

Methods

Information concerning experimental animals and ethical statement is available at SI 6.

Experimental design

After acclimatization for one week, adult (12-wo) virgin SHR females were randomly divided into two experimental groups: SHR and SHR-TAU. SHR-TAU dams received taurine (3 g/l) in drinking water (n= 12) from the time point of mating to the end of the lactation period. Untreated SHR (n= 10) and WKY rats (n= 8) were used as controls. Dams were housed in individual cages except during mating. Water intake was monitored across pregnancy and lactation periods in order to ensure that the daily drug intake was consistent across each pregnant rat (SI 7).

Pups were culled to 6-8 after birth. Offspring's body weights were measured daily until weaning and weekly until termination of the experiment. SBP was measured once a week by indirect tail-cuff method using a sphygmomanometer as previously described(61). The first measurement was performed at 13-wo after a period of 4 weeks of acclimation. Each weekly value corresponds to at least 3 independent measurements taken in a 5 minute period. Rats were intensively handled and trained in order to reduce stress as much as possible. Fasting animals were euthanized at 4 or 22 weeks via cervical dislocation and decapitation. Right kidneys were isolated, dried and weighed to calculate the kidney weight / body weight ratio. Heart was also excised and weighed and the cardiac hypertrophy index was calculated dividing the heart weight by the body weight in each animal. The left ventricle was isolated leaving the interventricular septum as an integral part of the left ventricle. The aortic arch, the iliac and mesenteric arteries were also excised and cleaned of adhesive fat and connective tissue. All tissues were quickly snap-frozen and stored in −76◦C until nucleic acid extraction. A portion of the thoracic aortic was fixed in 10% formalin for morphometry analysis.

Aortic morphometry analysis

Fixed thoracic aortic segments were embedded in paraffin and cut into traversal 5 μm-thick slices that were stained with hematoxylin and eosin. Images were obtained with a microscope (Olympus BX50) coupled to an adapted camera (Olympus DP50). Morphometrical analysis was performed by two different blinded operators using Scion Image 4.2 for Windows software. Three slides with 5 semi-serial (1 section every 25 μm) sections each, i.e. a total of 15 sections, were obtained from each sample. Analysis and calculations included the determination of lumen, CSA, wall thickness (Wt) and the ratio of the wall thickness to the lumen diameter (Wt/lumen).

RNA preparation and Real Time PCR for quantitative assessment of mRNA expression

We investigated mRNA expression of genes encoding Bcl-2, Bax, p53, eNOS, SOD2, SOD3, catalase, Tert and p16Ink4a. Total RNA was extracted with the use of the modified phenol extraction step method of Chomczynski and Sacchi(62). The RNA pellets were re-

suspended in RNase-free water, and the RNA concentration was quantified by measuring the absorbance at 260 nm in a nanodrop (Nanodrop ND-1000, Thermo Fisher Scientific Inc.). cDNA synthesis was performed by RT-PCR using 1-3µg of mRNA template, the Moloney Murine Leukemia Virus (Easy Script Reverse Transcriptase M-MLV, RNAse H-) Reverse Transcriptase and oligo(dT) primers according to manufacturer's protocol. PCRs were performed in a 7500 Real-Time PCR System (Applied Biosystems) using the KAPA SYBR® FAST qPCR Kit Master Mix (2×) Universal (Kapa Biosystems) and following the manufacturer's instructions. Each RT-PCR quantification experiment was performed in duplicate and all samples were tested blind to the experimental groups. The relative abundance of the target gene mRNA was normalized to the amount of a housekeeping gene [beta-2 microglobulin (B2m)] to carry out comparisons between the groups SI 8A. Specific primers are shown in SI 8B.

DNA isolation and measurements of relative telomere length

An assay based on real-time quantitative PCR was used for RTL measurement based on a method that uses a ratio of telomere DNA signal intensity to single-copy gene signal intensity (SI 8C)(63). DNA was isolated from frozen tissues using a sequential nucleic acid extraction method developed in our lab (full protocol available under request). DNA concentration was measured with a spectrophotometer and samples were diluted to a concentration of 10 ng/µL. mtDNA content was also estimated as described in SI 8D.

Statistical analysis

Our primary outcomes were: 1) change in SBP, 2) change in cardiac hypertrophy, and 3) change in aortic morphometry between SHR and SHR-TAU. Sample size was sufficient to detect a 12% difference with 80% power. Values are expressed as mean ± SEM to estimate the population mean from the sample data. All statistical analyses were carried out using the Statistica program package V7.0. Analyses were performed separately in each sex. Bartlett test was used to determine the homogeneity of variances and the Kolmogorov-Smirnov normality test was used to examine if variables were normally distributed. One-way analysis of variance (ANOVA) was employed to compare the means of three or more independent data sets. Planned (a priori) comparisons of Least Squares means were also performed.

The nonparametric Kruskal-Wallis test was used when the assumptions of normality of ANOVA were not met. For SBP analysis, the effects of blood pressure and the effects of perinatal taurine were separately analysed by the Kruskal-Wallis ANOVA test and ANOVA for repeated measurements respectively. A nominal significance threshold of P= 0.05 (two-sided) was set for all analyses(64).

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Table 1 Morphometric parameters in the thoracic aorta at 22-weeks old

	males			females		
	SHR	SHR-TAU	WKY	SHR	SHR-TAU	WKY
Lumen	1629.00±17.80	1581.74±14.19	1526.03±54.35 *	1399.86±20.89	1357.55±18.73	1267.19±34.49 **
Wt	152.08±2.54	142.11±4.78	95.20±7.94 **	118.92±2.49	120.76±3.76	88.93±1.59 ***
Wt/lumen	9.34±0.15	8.99±0.31	6.31±0.73 ***	8.5148±0.27	8.90±0.23	7.032±0.19 ***
CSA	859.00±24.29	785.69±26.41 #	502.16±26.19 ***	583.99±12.95	568.21±22.56	388.21±12.89 ***
CSA/BW	2.16±0.05	1.88±0.05 **	1.22±0.06 ***	2.49±0.09	2.38±0.09	1.70±0.10 ***

Footnote: Lumen (μm), wall thickness (Wt, μm), Wt/lumen, cross-sectional area x1000 (CSA, mm^2) and CSA/ body weight (CSA/BW, mm^2/mg) in SHR, SHR-TAU and WKY are shown as mean ± SEM. Planned contrasts (vs. SHR) showing differences are indicated as follows: * P<0.05, ** P<0.01, *** P<0.001. # indicates P=0.06. N male / female offspring: 5 / 5 SHR, 5 / 6 SHR-TAU, 4 / 4 WKY.

Figure legends

Figure 1 Systolic Blood Pressure

Footnote: SBP was registered from week 13 after a period of acclimation in SHR (circle), SHR-TAU (square) and WKY (triangle). Planned contrasts (SHR vs. SHR-TAU) showing differences are indicated as follows: * P<0.05 and ** P<0.01. Data are plotted as mean ± SEM. N male / female offspring: 7 / 7-9 SHR, 8-9 / 10-11 SHR-TAU, 9 / 6-7 WKY.

Figure 2 mtDNA content in the left ventricle

In Figure: mtDNA in SHR (black), SHR-TAU (grey) and WKY (white) is plotted as the mean ± SEM. Analyses were performed separately in each sex. Planned contrasts (4 vs. 22-wo) showing statistical differences are indicated with an asterisk. Significant level was taken at P<0.05. N 4-wo offspring: 10 SHR, 9 SHR-TAU, 8 WKY. N 22-wo offspring: 9 SHR, 9 SHR-TAU, 10 WKY.

Figure 3 Relative telomere length

Footnote: Relative telomere length (RTL) in SHR (black), SHR-TAU (grey) and WKY (white) is plotted as mean ± SEM. Planned contrasts (4 vs. 22-wo) showing statistical differences are indicated with an asterisk. N male / female 4-wo offspring: 7-8 / 6-8 SHR, 10 / 9-10 SHR-TAU, 5-7 / 6-7 WKY. N male / female 22-wo offspring: 6-7 / 7-9 SHR, 7-8 / 9-11 SHR-TAU, 9 / 6-7 WKY.

Figure 4 Gene expression in the aortic arch

Footnote: The data in SHR (black), SHR-TAU (grey) and WKY (white) is plotted as mean ± SEM. Contrasts (vs. SHR) showing differences are indicated as follows: * P<0.05, ** P<0.01, *** P<0.001. N male / female 22-wo offspring: 5-7 / 5-7 SHR, 6-8 / 5-9 SHR-TAU, 5-9 / 5-7 WKY.

Figures